ANALYSIS OF THE FRUIT OF RHAMNUS FRANGULA

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY J. LOWE HALL

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ANALYSIS OF THE FRUIT OF RHAMNUS FRANGULA.

Introductory and Historical.

While this work has to do only with the fruit of Rhamnus Frangula, no consideration of the subject could be complete without some mention of the other parts of the plant as well as some of the more closely related species. For the use of the various parts of these plants in medicine has a very ancient origin, and has been of prime importance in its chemical history and economic application. The writer is indebted to an article written by E. N. Gathercoal (1) in the Journal of the American Pharmaceutical Association from which the following brief historical consideration is taken.

For centuries the bark of a wild shrub, known in England as Alder Buckthorn or Berry Alder, has been used in Europe as a purgative. This bark is now recognized in most of the leading pharmacopoeias of the world, under the name of Frangula, or Frangulae Cortex. Rhamnus Frangula, the plant yielding the drug, ranges along roadsides and in thickets over all of Europe, except in the very northernmost parts, and east over northern Asia.

Associated with Rhamnus Frangula is Rhamnus Catharticus, a thorny shrub, named in England, Buckthorn or Waythorn. This plant is also found in northern Africa, India, and eastern

United States. The fruit, especially, has been employed for many centuries in Europe as a cathartic. It is now official in a few of the European pharmocopoeias. As a medicine the fresh, ripe berries are made into a decoction or the abundant juice is expressed and made into a syrup. The bark, also, possesses purgative properties, which, in the fresh bark, are said to be more drastic than Frangula.

Another group of Rhamni furnishing medicinal barks, is found along the western coast of North America. With the settlement of California by the Spaniards, the new-comers noted that the native Indians used the bark of a certain kind of a shrubby tree as a cathartic. The Spaniards named this plant and its bark Cascara Sagrada (Sacred Bark). This drug is now official in nearly all the pharmacopoeias of the world. It is obtained from the plant Rhamnus Purshiana, which ranges over the west slopes of the Cascade Mountains, from central California well up into British Columbia, and forms extensive low forests on the valley and mountain sides.

The term "ramnos" used by the early Greek physicians and naturalists, is thought to be derived from the Celtic "ram", signifying a tuft of thorns or branches. The name was applied to certain thorny plants by these writers, but from their meager or inaccurate descriptions it is impossible to establish that the plants mentioned by them were any of the Rhamni as we know them today.

The early Anglo-Saxons were acquainted with purgative properties of, at least, the Rhamnus Catharticus, for we find

the plant mentioned in their medical writings before the Norman Conquest. The juice of Waythorn berries is described as an aperient by Welsh physicians at the beginning of the thirteenth century.

Crescentius (1305) mentions Rhamnus Catharticus under the name Spina Cervinae and describes Rhamnus Frangula under the name Avornus, mentioning the use of the middle bark as an evacuant.

It is not until Matthiolus (2) (1548) in his commentary on the materia medica of Dioscorides, that a good description of Rhamnus Frangula, with mention of the purgative property of bark and berries, is found in literature. He first uses the name "frangula" in connection with the plant: (frango, frangere, meaning "to break", an allusion to the soft and fragile nature of its wood).

By 1700 the botanical characters of most of the European Rhamni were well established. Linnaeus (3) (1753) places them in the Petandria Monogynia. He includes both Rhamnus Frangula and Catharticus as natives of Sweden in his Flora Svecica (1745), and mentions as pharmaceutical products derived from them: Spina Cervinae Baccae, Syrupus Domesticus, Frangulae Cortex.

The bark of Rhamnus Frangula has been recognized in the pharmacopoeias of central Europe since the middle of the last century, including the Danish (1868), Norwegian (1870), Swedish (1871), German (1870), Prussian (1862), Hanoverian (1861), and Dutch (1871); Austrian (1889), French Codex (1908), U. S. (1880), and British (1885, though it was omitted from the last edition).

The chemistry of rhamnus barks presents much of interest

because from the first analysis by Gerber in 1828, it has been observed that the active principles are resinous in nature, difficult to separate from one another and to determine their true constitution. Even at the present day these analyses are far from being in a satisfactory condition.

Gerber, (4) obtained, among numerous other vegetable constituents, 2.7% of yellow resinous coloring matter and 4.6% of bitter acrid extractive, which he considered contained the active constituents. He noted the yellow coloring matter became dark-red with alkalies.

Hubert (5) (1830) analyzed the juice from the fruit of Rhamnus Catharticus. He found a bitter substance, apparently the active constituent, and closely resembling the cathartin of senna leaves, a green coloring matter, which in the ripe fruit is purple red, due to the action of acids in the ripening fruit, and a brown material insoluble in alcohol but easily soluble in water.

Fleury (6) (1842) obtained from the unripe berries of Rhamnus Catharticus, rhamnine in pale yellow crystals.

Winckler (7) (1849) obtained rhamnine from the unripe berries of Rhamnus Catharticus and cathartin from the ripe fruit. He considered that rhamnine by the ripening process is converted into cathartin and glucose. (This is the first published evidence of the glucosidic nature of these resinous constituents of the Rhamni).

Binswanger (3) (1849) found in frangula bark the crystallizable yellow coloring principle which was named (by L. A. Buchner) rhamnoxanthin, an ether-soluble amorphous resin, one or more alcohol soluble resins, a bitter substance of resinous nature in which the purgative properties of the bark seem to lie, sugar, gum, tannin, plant acids, extractive, etc. He compared the bark of Rhamnus Catharticus with Frangula bark and found that the constituents were similar, but included also a bitter, water soluble, crystallizable substance to which he attributed the greater hydragogue properties of the Rhamnus Catharticus bark. This principle was differentiated from the cathartin of senna leaves and named rhamno-cathartin. He found rhamnoxanthin also in the seeds of Rhamnus Catharticus and Rhamnus Frangula. The juice of the ripe berries contained a violet coloring matter turning red with acids and green with alkalies, a bitter extractive, etc. The unripe berries contained only the rhamnin of Fleury.

Buchner (9) (1853), who worked with Binswanger at Munich in 1849, obtained from the root bark of Rhamnus Frangula, rhamnoxanthin in sublimable, golden-yellow needles, very slightly soluble in water, but easily so in alcohol or ether (especially hot), readily in solutions of ammonia and the fixed alkalies with a fine purple-red color, and in concentrated sulfuric acid with a red color. By neutralization of the alkaline solution, the rhamnoxanthin was thrown out as a yellow powder, and by dilution of the concentrated sulfuric acid solution with water it was likewise separated out.

Casselmann (10) (1857) obtained the resinous constituent of Frangula in crystalline form, designated it frangulin, and decomposed it with the formation of glucose and an acid product he named frangulinic or nitro-frangulic acid.

Phipson (11) (1858) found rhamnoxanthin in the branches of Rhamnus Frangula and of Rhamnus Catharticus and corroborated Buchner's description of it.

Kubly (12) (1866) separated from frangula bark the glucoside, which he named avornin, an amorphous resin, and a principle similar to cathartic acid, which he had a short time previously isolated from senna leaves. The avornin he split into avornic acid and glucose.

Faust (13) (1869) stated that the frangulin of Casselmann, and the avornin of Kubly are identical, and assigned them the formula $C_{20}H_{20}O_{10}$. He named the acid resin from the decomposition of this glucoside, frangulic acid.

Liebermann and Waldstein (14) (1876) identified emodin (trioxymethylanthraquinone) from frangula bark and stated that frangulic acid is probably emodin.

Prescott (15) (1879) was the first to analyze cascara sagrada bark. He found a brown resin of strongly bitter taste, colored a vivid purple-red by potassium hydrate solution, sparingly soluble in water or ether, but freely so in alcohol, chloroform, benzol, carbon disulphide, and solutions of caustic alkalies, though precipitated from the latter by acids. He found also some other resins, tannin, oxalic and malic acids, etc.

Schwabe (16) (1888) found frangula to yield frangulin 0.04%, and emodin 0.1%. He corroborated the physical characters of frangulin as stated by Casselman and Faust, and amplified on them. His proximate analysis indicated the formula $C_{21}H_{20}O_9$. Frangulin by hydrolysis, yields emodin. He found in cascara bark emodin but no frangulin.

Thorpe and Miller (17) (1892) corroborated Schwabe's formula for frangulin and determined that the sugar from the decomposition of frangulin was a true rhamnose.

Cabannes (18) (1895) reported that in sections of frangula bark treated with alcoholic potassa solution, the parenchyma of the cortex, medullary rays, and bast, all acquire a strong red color, but that in cascara bark sections only one or two layers of the cortical parenchyma, the medullary rays, and the five or six inner rows of bast parenchyma take the color. Ammonia and soda solutions react the same as potassa.

Oesterle (19) (1899) found that frangula-emodin differs from aloe-emodin.

Perrot (20) (1900) stated that powdered frangula bark with alkalies produces a deep red color, but that powdered cascara bark gives a yellow color, and that the powders could be distinguished in this manner.

Tschirch and Polacco (21) (1900) analyzed Rhamnus Catharticus fruit and determined the presence of emodin, several coloring matters, a sugar, etc. The purgative action was ascribed to the emodin.

Tschirch and Pool (22) (1908) found that the emodins from frangula and cascara barks were identical, that neither of the barks yielded rhein, but that chrysophanic acid was present in frangula bark.

Schmidt (23) (1912) describes frangulin (rhamnoxanthin), ($C_{21}H_{20}O_9$), as occuring in lemon-yellow, glistening fine needle-crystals, odorless and tasteless, melting at 228° to 230° C. It is almost insoluble in water and in cold ether, but soluble in 180 parts of 80% hot alcohol. Concentrated sulfuric acid dissolves it with a dark-red color and with caustic alkalies it forms solutions of a purple red color. By boiling with an alcoholic solution of hydrochloric acid it becomes converted into rhamnose and frangula-emodin, ($C_{15}H_{10}O_5$), which forms bright red glistening needles melting at 255°C. It is insoluble in water, slightly soluble in alcohol, and easily soluble in chloroform and benzol.

The Material.

The berries of Rhamnus Frangula were gathered as they became ripe and immediately immersed in 95 per cent alcohol, thus preventing any enzymatic action. About a half liter of the berries covered with alcohol was available for this analysis. Consequently the work was handicapped considerably in attempting to purify and identify small amounts of material.

Method of Procedure.

The alcoholic extract was filtered from the berries and the berries washed with about 200 c.c. of alcohol (till the filtrate was colorless). The extract was brownish-black by reflected light, and when quite shallow transmitted a dull, dark-red light.

The berries were then dried in a vacuum oven at 70°- 80°C, and mulled in a mortar in order to break the hardened skins from the seeds. The skins were winnowed from the seeds and exhausted with boiling alcohol, which was then combined with the first extract.

This combined alcoholic extract was evaporated to a thick syrup consistency, and to it was added 300-400 c.c. of water. This mixture was placed in a refrigerator for preservation while the water-soluble material was going into solution. The gelatinous precipitate was filtered out, washed with water, and again taken up in alcohol, although an insoluble residue remained, as was often the case in similar subsequent operations; it may have been the result of resinification, hydrolysis, or similar reaction. Sufficient alcohol was added to the aqueous solution for preservation.

The dry seeds (to be analyzed later) were put in a dry stoppered flask.

A. The Water-Insoluble Fraction.

The accompanying diagram illustrates the procedure followed in manipulating the alcoholic extract, the Roman numerals to be used later in referring to the individual portions in their subsequent separations.

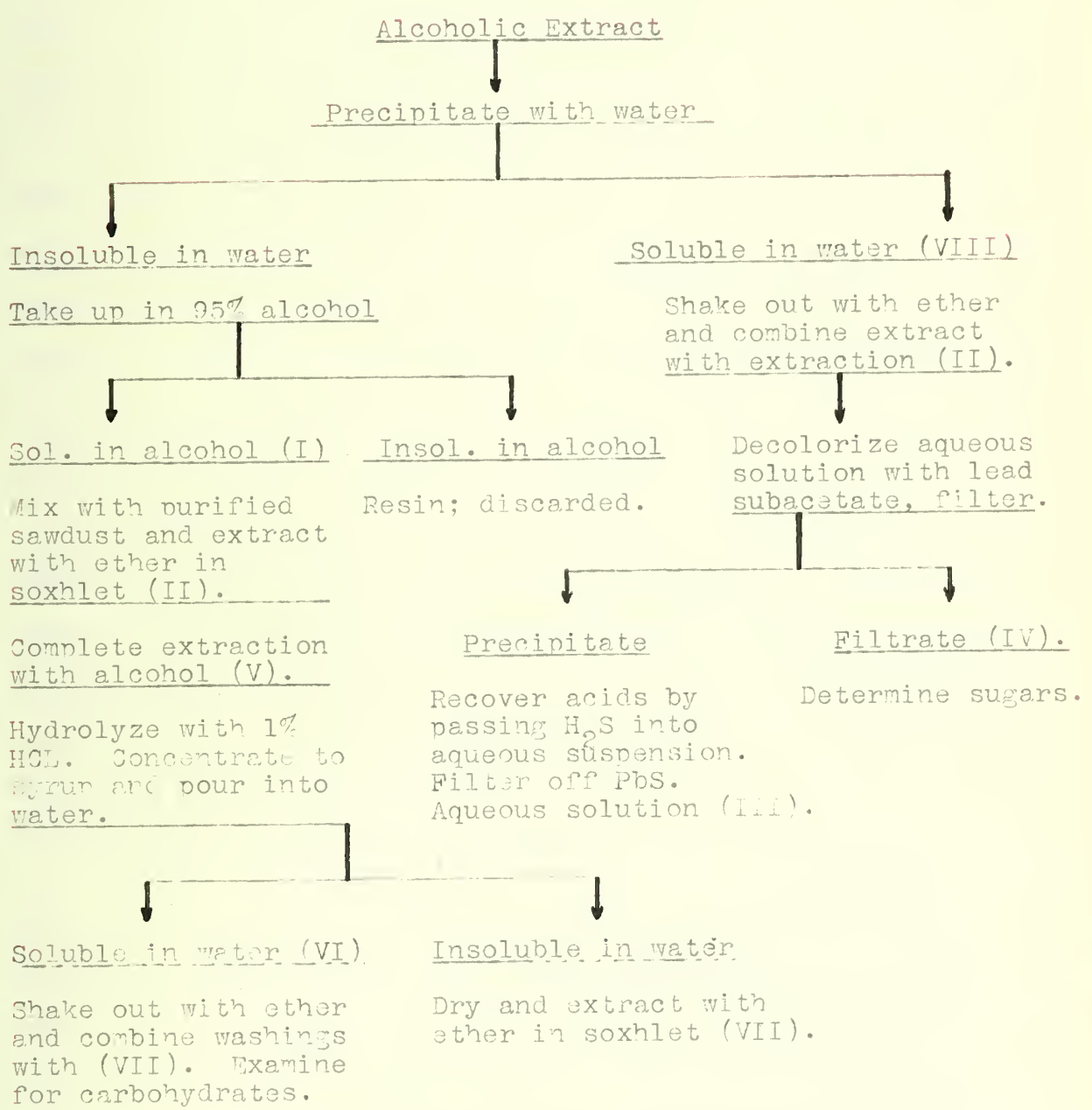
The water-insoluble portion of the original extract having been taken up in alcohol as previously mentioned, was then mixed with a sufficient quantity of purified sawdust to absorb it effectively, allowed to dry until the odor of alcohol was no longer apparent, and then placed in a large Soxhlet extraction apparatus. The material was then extracted with ether until it came through colorless (II, see diagram). Extraction was then completed with alcohol (V, see diagram).

The ether extract (II) gave a strong color test (red) with ammonia, and was therefore shaken out successively with 5 per cent solutions of ammonium carbonate, sodium carbonate, and sodium hydroxide; these fractions were acidified and shaken out with ether.

Fraction	Vol. of base required	Color	Comparative precipitation
Ammonium carbonate	75 c.c.	Faint pink	None
Sodium carbonate	230 c.c.	Very deep red	2
Sodium hydroxide	200 c.c.	Pale red	1

The precipitates from the second and third fractions, (the first was discarded), were soluble in glacial acetic acid, but did not crystallize out on concentration, a small fraction merely separating in a semi-gelatinous state. This material

DIAGRAM SHOWING MANIPULATION OF COMBINED ALCOHOLIC EXTRACT.



Test small amount of each ether extract for anthraquinone derivatives with a little ammonia (deep red or purple).

in each case was filtered off on a hard filter and dried, but amounted practically to nothing. The acetic acid solution was a very deep, rich red color, nearly opaque. The addition of water to each of these acetic acid solutions caused the precipitation of a bright yellow substance in a flocculent state, which was filtered out, redissolved in 5% sodium hydroxide, acidified, and shaken out with ether. On allowing the spontaneous evaporation of the ether solution of the sodium carbonate fraction on a large watch glass, it was noticed that the material appeared to be deposited in two forms, an outer ring of a deep red leaf-like material, and an inner circle of small yellow particles, - mostly the former.

The material in each case was again taken up in glacial acetic acid and concentrated on the steam bath. During this purification, the sodium hydroxide fraction decreased markedly in mass, and the acetic acid solution, even when concentrated to about 1 c.c., yielded only a few particles of apparently flocculent, non-crystalline material.

However the sodium carbonate fraction under similar treatment yielded a small crop of very finely divided material, melting at 250°- 265° C. An attempt to prepare an acetyl derivative yielded barely enough material of questionable nature for a melting point determination: it did not melt under 320°C.

From these data it is evidently impossible to arrive at any definite conclusions concerning the identity of the substances present, but it seems probable that the sodium carbonate

fraction, at least, contained emodin (red; melts at 255°C), and the sodium hydroxide fraction possibly chrysophanic acid (yellow, though melting much lower, 198°C, and, according to Beal and Okey (24), insoluble in cold solutions of alkali carbonates, but soluble in sodium hydroxide).

The alcoholic extract (V, see diagram) was hydrolyzed 3-1/2 hours on the steam bath with 100 c.c. 1% hydrochloric acid. It was then evaporated to a syrup, diluted with considerable water, and the precipitate filtered off and dried. The water solution was pale yellow. The dry precipitate was extracted with ether in a Soxhlet apparatus. The aqueous solution (VI) was shaken out with ether, the extract being combined with the Soxhlet extract (VII). The aqueous solution did not reduce Fehling's solution. The Molisch α-naphthol test for carbohydrate (Sherman (25) p. 57) was negative in comparison to a blank on distilled water. Therefore, as the subsequent examination of the ether extract (VII) showed considerable anthraquinone derivative, it must have been linked in some form insoluble in ether, but not with a carbohydrate.

The ether extract (VII) gave a very pronounced color test with dilute alkali. Accordingly it was extracted with the usual sequence of dilute bases.

Fraction	Vol. of base required	Color	Comparative Precipitation
Ammonium carbonate	150 c.c.	Pale yellow	Trace
Sodium carbonate	450 c.c.	Very deep red	Very heavy
Sodium hydroxide	100 c.c.	Pale red	Trace

The sodium carbonate fraction contained perhaps 0.2 gram of precipitated material, which was crystallized from hot glacial acetic acid, yielding a small crop of minute crystals, which appeared under the microscope as broad, ragged plates, and which melted at 252° - 255° C. They were deep red in color, but reflected a yellow fluorescent color similar to "fool's gold". The substance was evidently emodin. The other two fractions yielded negligible quantities of precipitate.

B. The Water-Soluble Fraction.

The aqueous solution (VIII) was shaken out with ether, but the extract gave no color test with alkali. The ether was evaporated on a large watch glass, leaving a thin film of light-yellowish brown material possessing a pleasant odor resembling peaches, and a bitter taste. It had the appearance of a gum and did not reduce Fehling's solution before or after hydrolysis, was insoluble in water, hot ten per cent sodium hydroxide, and acetic anhydride.

The aqueous solution (VIII) was treated with lead subacetate to remove its deep color, the precipitate filtered off, and hydrogen sulfide passed into the filtrate to remove the excess lead. After the lead sulfide was filtered off, the filtrate was colored only slightly yellow. This solution (IV) was warmed gently on the steam bath and air bubbled through it to remove the hydrogen sulfide.

The Molisch α-naphthol test gave decided indication of carbohydrate in the solution even to the extent that considerable precipitate was formed. The solution exerted a very marked reduction on Fehling's solution, and formed an osazone in 8-9 minutes which under the microscope appeared as the sheaves of long yellow needles characteristic of glucosazone; it melted at 198-202° C. Consequently the sugar might be either glucose or levulose. However, Seliwanoff's test for levulose (Hawk (26) p. 35) was negative; therefore there must have been glucose present in appreciable quantity. A very brilliant red anilin acetate test for furfural was obtained, indicating a pentose (Sherman (25) p. 57).

No positive test for rhamnose was obtained by the alcohol-sulfuric acid method (Browne (27) p. 377).

The lead subacetate precipitate was pulverized, suspended in water, and hydrogen sulfide passed in until the lead was converted to the sulfide, thereby liberating the organic acids carried down by the subacetate. Tannic, malic, and a trace of succinic acids were found by a system of analysis according to Barfoed (23). Briefly Barfoed's method is as follows:

Neutralize the solution with ammonia, concentrate to small volume, neutralizing again if necessary. Mix with 7-8 volumes of alcohol and allow to stand 12-24 hours; filter. The precipitate contains oxalic, tartaric, and citric acids; malic acid will appear in the filtrate and may be precipitated with lead subacetate. Tannic acid is mostly precipitated immediately

in slightly ammoniacal solution by calcium chloride. Lead subsuccinate is soluble in hot water, the submalate is not. Or treat the alkali salts of succinic and malic acid with lead subacetate till precipitation is complete, add ammonium acetate until precipitate dissolves, and add two volumes of alcohol; the lead malate precipitates, and the succinate remains in solution. Calcium malate is insoluble in a 50-70 per cent solution of alcohol (by volume) in water. Neutralized benzoic, acetic, and formic acids do not precipitate on addition of calcium chloride and 1-2 volumes of alcohol.

C. The Seeds.

The seeds are dicotyledonous, the cotyledons being flat and round, and lying parallel to the flat side of the seed; ordinarily two seeds, sometimes three, occur in each berry and are flattened against each other. A thin cross-section of a cotyledon was mounted on a slide and treated with 5% sodium hydroxide. Under the microscope a row of red spots appeared along the axis of the mount, which followed the location of the vacuoles, thus demonstrating the distribution of the anthraquinone derivatives in the seed.

The dry seeds were ground up in a coffee mill but with considerable difficulty caused by clogging; their oil content was such as to allow the ground seed to cohere tightly together. Thirty-two grams of the ground seed were obtained: it possessed a pleasantnut-like odor, and was of a dark chocolate brown color.

This material was extracted in a Soxhlet apparatus with ether. The extract gave a deep red coloration with dilute alkali, and was shaken out with dilute sodium hydroxide, in order to get an approximate idea of the total anthraquinone-derivative content. The alkali extract was acidified with dilute hydrochloric acid, the yellow precipitate filtered off, carefully dried and weighed; it amounted to 0.6 gram, or about two per cent of the weight of the dry seeds. This material would not again go entirely into solution in ether, chloroform, or mixtures of both, or in benzene. This conduct was noted at other times when the material was allowed to dry and warmed below 100° C. Apparently some constituent of it oxidizes readily. However the material seemed most soluble in benzene, and in such solution was shaken out with the usual succession of dilute bases.

Fraction	Vol. of base required	Color of Extract	Color of Benzene	Comparative precipitation
Ammonium carbonate	150 c.c.	Pink	Red	trace
Sodium carbonate	200 c.c.	Rich red	Yellow	1
Sodium hydroxide	225 c.c.	Dark red	Colorless	2

The first fraction was discarded. The second fraction failed to crystallize from hot benzene or alcohol, merely separating as a small amount of bright yellow amorphous material.

The third fraction likewise would not crystallize from benzene or alcohol. During the evaporation of the alcohol from this fraction on the steam bath, the material, on being exposed to the air, even before all the alcohol was gone, turned black

and acquired a sticky consistency. It would not then completely redissolve in alcohol, a black sediment remaining. The solution was decanted from the sediment and allowed to evaporate spontaneously; the deposit was dark purplish brown. This experience serves to show how carefully the material must be handled to prevent oxidation.

The remaining ether extract of the seeds was evaporated on the steam bath, leaving a clear mobile, pale-yellow oil possessing the pleasant odor characterizing the ground seeds. This oil was dried in vacuo over concentrated sulfuric acid, and the following constants determined:

The index of refraction was taken by an Abbe refractometer at 19° C. and corrected to 15.5° C.

Reading at 19° C - - - - - - - - - 1.4726

Reading corrected to 15.5° C - - 1.4749

The specific gravity was determined with a Westphal balance by the alcohol-water method. A few drops of the oil were put in a cold mixture of alcohol and water, and the mixture adjusted by adding alcohol or water until the droplets of oil remained stationary in suspension at 15.5° C. At this point the specific gravity of the mixture was read on the Westphal balance.

Specific gravity of oil at 15.5° C - - - 0.9133

The iodine number was determined according to the method of Wijs. The iodine monochloride solution in glacial acetic acid was added to the sample (0.2 - 0.4 gram) dissolved in chloroform, and contained in a glass stoppered flask. After standing thirty minutes a solution of potassium iodide was added,

and the free iodine titrated with standard sodium thiosulphate solution. A blank determination was made at the same time.

Thiosulfate solution, 1 c.c. = 0.01376 gram iodine.

	I	II
Weight of sample	0.2382	0.2932
Thiosulfate titration		
Blank	47.00 c.c.	47.00 c.c.
Sample	26.78	22.18
Net	20.22	24.82
Iodine absorbed	0.2780 g.	0.3415 g.
Iodine number	116.7	117.1

The saponification number was determined by refluxing the sample with alcoholic potash on a steam bath for thirty minutes, cooling, adding phenolphthalein, and titrating with standard acid. A blank determination was also made.

Normality factor of acid 0.1003.

	I	II
Weight of sample	2.1132 g.	2.0137 g.
Acid titration		
Blank	97.81 c.c.	97.81 c.c.
Sample	25.00	28.50
Net	72.81	69.31
Saponification number	193.9	193.7

The per cent soluble and insoluble acids were determined from the combined solutions from the saponification value determination. Two cubic centimeters more of standard acid

than required to liberate the fatty acids from the soap were added to the solution. The mixture was slightly warmed to facillitate cohesion of the insoluble acids, and transferred to a separatory funnel whereby the floating insoluble acids were separated and washed. The acids were transferred by the aid of a little ether to a small weighing bottle, placed in a vacuum desiccator over concentrated sulfuric acid, and dried to constant weight.

Weight of combined samples	4.1269 g.
Weight of insoluble acids	3.8712 g.
Per cent of insoluble acids	93.8

The percent soluble acids was determined by titrating the aqueous solution obtained above with standard alkali, allowing for the two cubic centimeters excess of standard acid used.

Normality factor of alkali 0.0998.

Weight of combined samples	4.1269 g.
Titration with alkali	4.90 c.c.
Excess standard acid	2.00
Titration of soluble acids	2.90
Calculated as butyric	0.02548 g.
Per cent soluble acids	0.62

The index of refraction of the insoluble acids was taken by an Abbe refractometer, and corrected to 15.5° C, as the acids solidify below 19° C.

Temperature - - - - - - -	24.4° C.	22.5° C.
Reading - - - - - - - - -	1.4653	1.4660
Corrected to 15.5° C - -	1.4635	1.4635

The melting point of the insoluble acids was taken by drawing the melted acids into melting point tubes about one millimeter in diameter, leaving in a refrigerator 24 hours, and melting in the usual way in an open beaker of water. The melting point was taken as the point at which the acids became transparent.

Melting point of insoluble acids - - - 26.5° C.

The solidifying point of the acids, or titer test, was taken by surrounding the beaker with ice-water and noting the point at which turbidity appeared.

Solidifying point of insoluble acids - - 19.5° C.

The iodine number of the insoluble acids was determined in the same way as for the oil.

Thiosulfate solution, 1 c.c. = 0.01376 grams iodine.

	I	II
Weight of sample	0.2176 g.	0.2246 g.
Thiosulfate titration		
Blank	46.90 c.c.	46.90 c.c.
Sample	30.65	29.90
Net	16.25	17.00
Iodine number	101.9	101.6

Because of the long period of time required to dry the insoluble acids, oxidation evidently took place, since the iodine number of the acids should be higher than that of the oil. The desiccator was evacuated with an efficient aspirator, and the precaution of drying in an atmosphere of carbon dioxide was not thought necessary.

The neutral equivalent of the insoluble acids was determined by dissolving the samples in neutral alcohol, adding phenolphthalein, and titrating with standard alkali.

Normality factor of alkali 0.0998.

	I	II
Sample	0.5365 g.	0.4086 g.
Titration	18.36 c.c.	14.65 c.c.
Neutral equivalent of insoluble acids	292.8	293.1

The oil is a characteristic seed-oil, resembling in these constants sunflower-seed oil, and cottonseed oil, but having a slightly lower specific gravity and less viscous behaviour.

Discussion of Results.

It is a notable fact that the sodium carbonate fraction of the alkali extract was distinctly the largest fraction in the case of the alcoholic extract and the hydrolyzed alcoholic extract, but in the case of the seeds the sodium hydroxide fraction was the largest. However, the solvent in the first two cases was ether, whereas benzene was used in the latter, which might explain the difference. But it seems likely that at least two types of alkali extractive materials were present, that probability being further strengthened by the fact that the sodium carbonate removed the red color from the benzene solution, leaving it a strong yellow, even though the sodium carbonate fraction was much smaller than the sodium hydroxide fraction.

It was found by experiment that a very small amount of emodin is sufficient to give a distinct red color to benzene. According to Beal and Okey (24) emodin is soluble in dilute sodium carbonate; chrysophanic acid is insoluble in sodium carbonate, but soluble in dilute sodium hydroxide. Hence it is probable that the emodin was removed by the sodium carbonate, and that the remaining material contained chrysophanic acid, which is yellow. Unfortunately the latter fraction was overheated or oxidized in evaporating the solvent, and identification was made impossible.

While the resinous material which was hydrolyzed probably contained frangulin, the aqueous solution could not be shown to contain rhamnose, or for that matter any reducing sugar, or even a carbohydrate. The solution contained sufficient alcohol for preservative and was kept in a stoppered flask until examined. Nevertheless this was the only case in which emodin was obtained in sufficiently pure state to permit crystallization. It is possible, during the concentration of the hydrolyzed solution for precipitation in water, that the small amount of hydrochloric acid present was sufficient to convert the rhamnose to methyl furfural, which would be lost by volatilization.

The oil obtained from the seeds has promising possibilities. As shown by its constants it resembles sunflower, maize, and cottonseed oils. (Sherman) (25).

	Rh.Frang. Seed Oil	Sunflower Seed Oil	Maize Oil	Cottonseed Oil
Saponification No. - - - - - -	193.8	188 - 196	188 - 194	190 - 197
Iodine No. - -	116.9	104 - 135	111 - 124	104 - 116
Spec. Gravity 15.5° C. - -	0.9183	0.920-0.927	0.921-0.926	0.920-0.925
Index of refr. 15.5° C. - -	1.4749	1.474-1.478	1.475-1.477	1.473-1.476

It seems reasonable to expect that the oil expressed or extracted simply from the ground dry seeds, and containing its normal content of the emodin, etc., would make an excellent lubricant and cathartic.

Summary.

The analysis of the fruit of Rhamnus Frangula indicates emodin and possibly chrysophanic acid, glucose, a pentose, a small amount of light brown gum, tannic acid, malic acid, succinic acid, a resin (which was probably frangulin, though no rhamnose was found), a deep purplish-red coloring matter soluble in alcohol, and a fatty oil constituting approximately eighteen per cent of the weight of the dry seeds.

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